time scale to study kinetics of lumirhodopsin decay and the effect of membrane environment on the first equilibrium constant, K_1, and on the pK_a of the second equilibrium. Reconstituted membranes of rhodopsin with POPC, DOPC, or a mixture of DOPC and DOPE were studied at 30°C. We also extended previous 20°C studies of the pH dependence of the equilibria in the native disk membranes, to determine how increased temperature affects lumirhodopsin decay through the purely transient 380 nm absorbing species, Meta I_{380}, into the final equilibrium mixture. Meta I_{380} has recently attracted substantial interest, since time-resolved circular dichroism measurements on the microsecond timescale suggest the chromophore has a different conformation than in later 380-nm photointermediates. Our results suggest SB deprotonation precedes other activating changes in the protein. Significant details are now emerging that give new insights into rhodopsin activation and complement FTIR and spin-label approaches.

1509-Pos

Multi-Scale Dynamics of Rhodopsin Activation as a Paradigm for GPCR Function

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G-protein coupled receptors (GPCRs) are membrane proteins that act as signaling cascade initiators responsible for a myriad of cellular processes. Ligand binding causes GPCR conformational changes that allow the receptor to interact with its cognate G-protein. Several techniques have structurally characterized rhodopsin photointermediates, but none have directly revealed the protein dynamics.Here we report a model whereby ps-ns ligand dynamics are coupled to the μs-ms protein motions during activation. These protein motions represent activated conformational substates on a hierarchy of time scales. Previous models propose that rhodopsin activation is a simple switch whereby retinal isomerizes from an 11-*cis* to an *all-trans* conformer, transforming from an inverse agonist to an agonist. In contrast, our model is motivated by FTIR and UV-visible results showing thermodynamic coupling to several substates in rhodopsin activation (metaI, metaII$_a$, and metaII$_b$) [1]. Furthermore, new ^{2}H NMR data from selectively labeled retinal ligands bound to rhodopsin are able to show that each retinylidene methyl group, especially the C9-methyl, acts as a dynamical hotspot in the activation pathway [2]. Relaxation times are fitted to three-fold jump and continuous diffusion models and correlated to methyl rotation rates in the different rhodopsin activation states, revealing distinct site-specific characteristics for each photointermediate. Recent solid-state NMR [3] and EPR [4] studies showed appreciable protein movements in the photointermediate pathway, further supporting our data. An activation mechanism emerges whereby conformational substates depend on a multivariate energy landscape encompassing retinal and protein dynamics as well as lipid bilayer interactions. [1] M. Mahlingam *et al*. (2008) *PNAS***105**, 17795-17800. [2] M.F. Brown *et al.* (2009) *BBA*, in press. [3] S. Ahuja *et al*. (2009) *J. Biol. Chem.***284**, 10190-10201. [4] C. Altenbach *et al*. (2008) *PNAS***105**, 7439-7444.

1510-Pos

Consequences of Fast, Stochastic Rhodopsin Shutoff for a Model of Phototransduction in Rods

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Rod photoreceptors signal the number and timing of photon absorption, a property that requires that each single photon response be of similar amplitude from trial to trial. How such reproducibility is achieved has been the subject of much experimental and theoretical work, which has demonstrated the importance of multiple steps in rhodopsin deactivation and diffusion of second messengers (Mendez et al., 2000; Bisegna et al., 2008). So far, all previous models have assumed that rhodopsin lifetime is significantly longer than recent measurements indicate (Krispel et al., 2006; Burns and Pugh, 2009). Additionally, recent biochemical studies have provided new details about the dependence of rhodopsin deactivation on phosphorylation level (Vishnivetskiy et al., 2007) that should inform a complete model of light response kinetics and reproducibility. We have implemented a spatio-temporal model of phototransduction in which the rhodopsin deactivation scheme is a stochastic multi-step process lasting no more than 50 ms. The parameters of this model were constrained using an extensive data set obtained from a variety of transgenic mouse lines, each developed to perturb rhodopsin activity, PDE deactivation, or Ca^{2+} feedback. Our simulations demonstrate the relative contributions of stochastic rhodopsin deactivation, Ca^{2+} feedback to guanylate cyclase, and second messenger diffusion to single photon response variability under biologically relevant constraints.

1511-Pos

Functional Structures of Photo-Activated Rhodopsin Disk Membranes Using Single Particle Tracking

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Heterotrimeric G-proteins interact with their G-protein coupled receptors (GPCRs) via key binding elements comprising the receptor-specific C-terminal segment of the alpha-subunit and the lipid anchors at the alpha-subunit N-terminus and the gamma-subunit C-terminus. Direct information about diffusion and interaction of GPCRs and their G-proteins is mandatory for an understanding of the signal transduction mechanism. By using fluorescence microscopy and single particle tracking we showed that the encounters of the alpha-subunit C-terminus with the GPCR rhodopsin change after receptor activation revealing inhomogeneous and restricted diffusion of the receptor (1). To obtain further information about the underlying membrane structure in the signaling state of rhodopsin we now constructed high-resolution transducin visits maps on rhodopsin disk membranes using the inherent information from the single molecule traces.

(1) Kim, T.Y., Uji-i, H., Moeller, M., Muls, B., Hofkens, J. and Alexiev, U. *Biochemistry* 48, 3801-3803(2009)

1512-Pos

Molecular Dynamics Simulations of Active Receptor-G Protein Complex in a Lipid Bilayer

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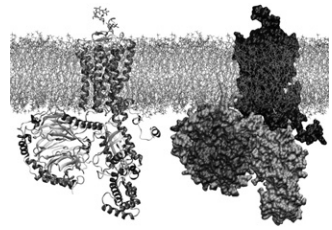

The crystal structure of opsin in its putative active state, the G-protein interacting conformation (Ops*-$G_t\alpha CT_{K341L}$), is arguably the most important breakthrough since the reports of structures for ground-state rhodopsin and the β_2-adrenergic receptor. We use this structure as a template and propose a structural model of the complex with full-length transducin (Ops*-$G_t\alpha\beta\gamma$) based on additional experimental structures including dark-state rhodopsin and holotransducin (Gt, $G_t\alpha\beta\gamma\bullet$GDP). We dock Gt with a reconstructed model of C-terminal α5 helix of $G_t\alpha$ to the open binding site in Ops*. Our model differs from others that propose a requirement for a 40°-tilt of $G_t\alpha\beta\gamma$ relative to the α5 helix in order to avoid steric clashes between $G_t\beta\gamma$ and the membrane. We further report a new method based on grid potentials to embed the complex into a POPC bilayer membrane. Compared with our previous molecular dynamics (MD) studies of the inactive states of rhodopsin[1] and β_2-adrenergic receptor,[2] these new simulations shed light on the role of the protonation state of the opsin residues K296(7.43) and E134(3.49) in stabilizing the receptor-G-protein complex. 1) T.Huber, et al. (2004) Biophys.J. 86:2078-2100. 2) T.Huber, et al. (2008) Biochemistry 47:11013-11023.

1513-Pos

Studying the Diffusion Characteristics of Different Activity States of the Human Adenosine-A3 Receptor Using Fluorescence Correlation Spectroscopy

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The adenosine-A_3 receptor is one of four known G-protein coupled receptors activated by the nucleoside adenosine. Here we use fluorescence correlation spectroscopy (FCS) in conjunction with pharmacological and molecular biology approaches to investigate the diffusion characteristics of different activity states of the human A_3-receptor. Initial FCS experiments using Chinese hamster ovary (CHO) cells expressing the wild type human A_3-receptor and the fluorescent adenosine receptor antagonist XAC-X-BY630 revealed both fast and slow moving complexes at the cell membrane, with average diffusion co-efficients of 1.58 ± 0.16 μm^2/s (τ_{D2}) and 0.081 ± 0.007 μm^2/s (τ_{D3}), respectively. At concentrations of XAC-BY630 ranging from 1-10 nM the amount of τ_{D3}, but not τ_{D2}, increased in a concentration-dependent manner. Pre-incubation of cells with the A_3-receptor specific antagonist MRS1220 at concentrations ranging from 0.3-300 nM significantly reduced the amount of slow moving (τ_{D3}) complexes in a concentration-dependent manner, indicating that they represent receptor bound ligand. Parallel experiments in which CHO cells were transfected with GFP tagged wild-type A_3-receptor (wt), a G-protein uncoupled mutant (W243A, W243F), or a constitutively active